INTERDEPARTMENTAL CORRESPONDENCE

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TO: T. Ha, Sponsor

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NOTEBOOK NUMBER: QH-1498

SUBJECT AND STUDY NO.: RAT GASTRIC LESION STUDY- Pepto-Bismol with Simethicone - ETOH Study - ANTR# 53

SUMMARY

The objective of this rat study was to determine if the addition of simethicone to Pepto-Bismol (as an anti-gas agent) would affect the protective effects of Pepto-Bismol against ethanol-induced gastric damage. Two formulations of Pepto-Bismol with simethicone were evaluated in this study. Pepto-Bismol without simethicone was used as a positive control. Water was used as a negative control.

Pepto-Bismol alone provided 68% protection against ethanol compared to the water control group. The Pepto-Bismol treatments with simethicone added provided 74-85% protection from ethanol. There were no significant statistical differences between any of the Pepto-Bismol treatments, with and without simethicone.

Simethicone alone did not provide any gastric protective effects. There were no significant statistical differences between simethicone and water in gastric lesion scores.

Results of this rat study indicate that the addition of simethicone to Pepto-Bismol will not adversely affect the protective effects of Pepto-Bismol on the gastric mucosa.

OBJECTIVE

The objective of this rat study was to determine if the addition of simethicone to Pepto-Bismol (as an anti-gas agent) would affect the protective effects of Pepto-Bismol against ethanol-induced gastric damage. Two formulations of Pepto-Bismol with simethicone were evaluated in this study. Pepto-Bismol without simethicone was used as a positive control. Water was used as a negative control.

MATERIAL AND METHODS:

Experimental Design:

Type of Study: Gastric Lesion

Species: Rat- Sprague-Dawley

Source: Charles Rivers Breeding Laboratories (Raleigh)

Sex: Male

Initial Weight: 185-196 grams

Average Weight of Rats on Study: 314 grams

Total Number of Animals Used: 60

Number of Animals per Group: 11-12

Means of Animal Identification: Cage card

Housing: 1 rat per cage. Stainless steel wire-bottom suspended cages with absorbent excreta paper beneath.

Site and/or Location: BETF 790 Building- Room A-109

Test Substances:

1) Pepto-Bismol Liquid Positive Control
   Dose Form: Liquid X Tablet___Capsule___Caplet___Powder___Slurry___
   Other_____
   Total Dose of Active per Animal: 34 mg bismuth subsalicylate
   Dose Level of Active in Each Tablet/Capsule, etc..: 14.7 mg/ml
   Number of Tablet/Capsules, etc.. per Animal: 2.31 mls
   Code or Lot Number/Expiration Date: HH-0736-18I
   Special Handling Requirements: Room Temperature
   Category: Drug/Food X Non-Food/Non-Drug___ Both___

2) Pepto-Bismol Liquid with Simethicone
   Dose Form: Liquid X Tablet___Capsule___Caplet___Powder___Slurry___
   Other_____
   Total Dose of Active per Animal: 34 mg bismuth subsalicylate
   Dose Level of Active in Each Tablet/Capsule, etc..: 19.3 mg/ml
   Number of Tablet/Capsules, etc.. per Animal: 1.76 mls
   Code or Lot Number/Expiration Date: HH-0736-18D
   Special Handling Requirements: Room Temperature
   Category: Drug/Food X Non-Food/Non-Drug___ Both___

3) Pepto-Bismol Liquid with Simethicone
Dose Form: Liquid X Tablet___Capsule___Caplet___Powder___Slurry___
Other_____
Total Dose of Active per Animal: 34 mg bismuth subsalicylate
Dose Level of Active in Each Tablet/Capsule, etc..: 18 mg/ml
Number of Tablet/Capsules, etc.. per Animal: 1.89 ml
Code or Lot Number/Expiration Date: HH-0736-18G
Special Handling Requirements: Room Temperature
Category: Drug/Food X Non-Food/Non-Drug___ Both___

4) Simethicone Diluted AF Emulsion - Dow Corning
Dose Form: Liquid X Tablet___Capsule___Caplet___Powder___Slurry___
Other_____
Total Dose of Active per Animal: 4 mg Simethicone
Dose Level of Active in Each Tablet/Capsule, etc..: 2 mg/ml
Number of Tablet/Capsules, etc.. per Animal: 2 ml
Code or Lot Number/Expiration Date:_____
Special Handling Requirements: Room Temperature
Category: Drug/Food X Non-Food/Non-Drug___ Both___

5) Deionized Water - Negative Control
Dose Form: Liquid X Tablet___Capsule___Caplet___Powder___Slurry___
Other_____
Number of Tablet/Capsules, etc.. per Animal: 2.31 mls
Special Handling Requirements: Room Temperature
Category: Drug/Food___ Non-Food/Non-Drug X Both___

Test Product Handling Requirements: All treatments were made up in advance and stored at room temperature. The treatments were mixed for approximately 30 minutes on a Magnestir mixer prior to and during dosing.

Route of Exposure: Oral gavage- Size Fr 8 stomach tube (Crocker Fels)

Test Methods:

The rats were randomly allocated into cages upon arrival to the facility, fed Purina Laboratory Rodent Meal, and given deionized water for a 7-day acclimation period preceding the study.

The rats were weighed 18-24 hours before dosing, food-fasted for 24 hours and water-fasted for approximately 2 hours before dosing.

Each rat was dosed with 1.6 mls of test material (5 ml/kg), followed in one-half hour by 1.6 mls of 100% ethyl alcohol (5 ml/kg). (Doses levels were based on the average weight of all of the animals.) The doses were given by oral gavage using size Fr8 nelaton stomach tubes and syringes (3cc) with syringe adaptors.

One hour after the ETOH dose, the rats were sacrificed by carbon dioxide asphyxiation and the stomachs were removed. The stomachs were cut along the greater curvature (cardia to pylorus), rinsed in tap water then rinsed in 0.9 percent saline solution. The stomachs were placed in pre-numbered vials containing approximately 10 mls 0.9% saline solution until graded.

Just prior to grading, each stomach was rinsed with tap water and spread on a white 3x3 card to expose the full gastric mucosa. The stomachs were graded using the Zidas (Zeiss) method of measuring lesion length $(mm)^2$. The sum of the lesion lengths of each rat represent the individual rat scores.

The rat scores were used for statistical analysis and comparison (Student's T-Test and ANOVA).

RESULTS:

Table I
Summary of Results

Treatment	Stomach Score X mm/length		% Gastric Protection from ETOH
Pepto-Bismol with Simethicone HH-0736-18D	15.6	s,w	85%
Pepto-Bismol with Simethicone HH-0736-18G	26.2	s,w	74%
Pepto-Bismol	32.7	s,w	68%
Water Control	102.2	d,p,g	---
Simethicone	135.6	d,g,p	---

d= significantly different from Pepto-Bismol/Simethicone HH-0736-18D at $\alpha < 0.05$.
g= significantly different from Pepto-Bismol/Simethicone HH-0736-18G at $\alpha < 0.05$.
p= significantly different from Pepto-Bismol at $\alpha < 0.05$.
s= significantly different from Simethicone at $\alpha < 0.05$.
w= significantly different from water at $\alpha < 0.05$.

As seen in Table I, the individual rat scores in Attachment I, and the ANOVA data analysis in Attachment II, rats given Pepto-Bismol liquid with and without simethicone demonstrated significantly lower stomach scores (15.6-32.7 mm), and greater gastric protection against ethanol (68-85%) than rats given simethicone alone and water (102.2-135.6 mm).

There were no statistical differences between the Pepto-Bismol treatments with and without simethicone in stomach lesion grades, and there were no statistical differences between simethicone alone and the water control groups in stomach lesion grades.

DISCUSSION:

Rats given Pepto-Bismol with and without simethicone produced gastric lesion scores between 15.6 and 32.7 mm. Pepto-Bismol alone provided 68% protection against ethanol compared to the water control group. Although the Pepto-Bismol treatments with simethicone added provided directionally higher gastric protection than Pepto-Bismol alone (74-85% protection), there were no significant statistical differences between any of the treatments.

Although the gastric lesion scores from rats given simethicone were directionally higher than those from rats given the water control, indicating more damage (135.6 vs. 102.2 mm), there were no significant statistical differences between the groups. These results indicate that simethicone has no gastric protective effects against ethanol-induced gastric damage.

CONCLUSION

Results of this rat study indicate that the addition of simethicone to Pepto-Bismol will not adversely affect the protective effects of Pepto-Bismol on the gastric mucosa.

M. F. Lukacovic, Study Director

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Attachment I - Student's T-Test
Attachment II - ANOVA Data Analysis

	Pepto-Bismol	ANTR# 53 Pepto/Simeth. HH-0736-18D	Pepto-Bismol with Simethicone Pepto/Simeth. HH-0736-18G	Simeth-icone	Water Control
	34.74	0.00	85.12	64.17	45.99
	51.85	1.58	36.57	172.40	206.80
	0.62	6.36	0.00	144.00	63.99
	10.90	12.12	35.52	99.61	107.70
	0.00	17.13	3.99	87.65	101.20
	8.67	9.48	1.28	223.40	57.83
	63.83	33.00	0.00	43.57	152.50
	2.20	0.00	0.00	147.10	0.90
	42.25	21.38	0.67	145.70	162.00
	38.00	21.75	82.87	224.00	154.60
	106.70	39.97	26.35	90.80	127.10
		24.13	41.71	184.90	46.01
mean	32.71	15.58	26.17	135.61	102.22
std	33.19	13.01	31.54	59.38	60.64
se	10.01	3.75	9.11	17.14	17.51
n	11	12	12	12	12

T-Tests	t value	p <	d.f.
Group 1 vs			
Group 2	1.603		21
Group 3	0.483		21
Group 4	-5.184		21
Group 5	-3.447		21
Group 2 vs			
Group 3	-1.076		22
Group 4	-6.840		22
Group 5	-4.839		22
Group 3 vs			
Group 4	-5.638		22
Group 5	-3.854		22
Group 4 vs			
Group 5	1.363		22

ONE WAY ANALYSIS OF VARIANCE

ANTR# 53 Pepto-Bismol with Simethicone

Group 1: Pepto-Bismol
Group 2: Pepto-Bismol with Simethicone HH-0736-18D
Group 3: Pepto-Bismol with Simethicone HH-0736-18G
Group 4: Simethicine
Group 5: Water Control

SOURCE	SUM OF SQUARES	D.F.	MEAN SQUARE	F RATIO
BETWEEN	135080.50	4	33770.1249	17.69
WITHIN	103059.72	54	1908.5134	
TOTAL	238140.22			

Bartlet's chi square test 1

COMPARISONS	(1=SIG, 0=NON-SIG)			
Group 1 vs Group 2	0	1	1684.2156	0.88
Group 1 vs Group 3	0	1	244.9824	0.13
Group 1 vs Group 4	1	1	60771.3442	31.84
Group 1 vs Group 5	1	1	27731.3923	14.53
Group 2 vs Group 3	0	1	673.8102	0.35
Group 2 vs Group 4	1	1	86448.0067	45.30
Group 2 vs Group 5	1	1	45042.2733	23.60
Group 3 vs Group 4	1	1	71857.5380	37.65
Group 3 vs Group 5	1	1	34697.9267	18.18
Group 4 vs Group 5	0	1	6689.4027	3.51

		D.F.		TABLE	F RATIO
T VALUE	2.0050	-			
		4	54	p = 5%	2.55
LSD	36.08	1	54	p = 5%	4.02
		4	54	p = 1%	3.71
		1	54	p = 1%	7.15

	1	2	3	4	5
Average	32.71	15.58	26.17	135.61	102.22
Std Dev	33.19	13.01	31.54	59.38	60.64
n=	11	12	12	12	12